REMARKS

This Reply is responsive to the Office Action dated March 1, 2000. Entry and consideration of the following amendments and remarks pursuant to 37 CFR §1.116 is respectfully requested.

At the outset, applicants note the amendments presented above. Claims 86-109 have been amended to replace the word "proliferating" with the word "non-quiescent." This amendment finds support in the disclosure at page 29, lines 4-5 and page 49, lines 20-22. In addition, claim 109 has been canceled in response to the Examiner's observation that claims 108 and 109 are duplicates. No new matter has been added.

Turning now to the Office Action, claims 86-109 were provisionally rejected under the judicially created doctrine of obvious-type double patenting as being unpatentable over claims 1-24 of U.S. Patent No. 5,945,577. Applicants again request that the rejection be held in abeyance until the indication of allowable subject matter, at which time applicants will gladly submit a terminal disclaimer if so required.

Next, claims 86-109 were rejected under 35 U.S.C. §112, first paragraph, because the specification, while being admittedly enabled for an improved method of cloning using nuclear transfer using as the donor cell or donor cell nucleus a proliferating somatic cell which has been expanded in culture, does not allegedly provide enablement for such a method using any proliferating cell. Essentially, in making the rejection the Examiner distinguishes between cells that are "proliferating" and cells, such as muscle or liver cells, that are considered to be terminally differentiated, and alleges that the specification has not described or contemplated the use of such terminally differentiated cells as nuclear donors in the claimed method. The Examiner supports her argument by noting that there must be

"some outside event or external stimulus" for the nucleus to re-program itself, and that because the specification allegedly fails to "point out any special treatment or feature of the disclosed protocol which is responsible for the reprogramming step" it can only be the expansion in culture that is responsible for the reprogramming. Applicants respectfully traverse the rejection.

First, Applicants respectfully note that the rejection appears to be based on a misunderstanding as to the meaning of the term "proliferating," which was clearly defined in the specification and does not equate to cell expansion in culture. For instance, as described on page 49, lines 20-22 of the specification, proliferating cells are merely those that are not synchronized at any one stage, and include any cell that has not been serum starved, or any cell which is not quiescent. The word "proliferating" was particularly chosen to distinguish the surprising nature of the present invention from the prior disclosure of the Roslin group, which alleged that serum starvation of the donor cell, i.e., as to produce a quiescent cell, was necessary for successful nuclear transfer (see page 28 of the specification, last paragraph). The term "proliferating" was never intended to refer only to cells that are expanded in culture, and applicants believe that this intention is clear when the entire specification is considered as a whole.

For instance, as discussed on page 28 of the specification, lines 12-22, fibroblast cells are considered ideal for use in the claimed methods because they can be obtained directly from developing fetuses and adult cows in large quantities. As also noted in this passage, fibroblasts were previously considered a poor cell type to be used in cloning procedures because they are differentiated. But as stated in the same passage, one point of novelty in the present invention is that differentiated cell types are used.

It is also clear from the cited passage on page 28 that one *advantage* of using fibroblasts for the present invention is that they can be clonally propagated, i.e., expanded in culture, which *facilitates* gene targeting procedures. However, such clonal propagation is at no point disclosed as being a necessity for the use of differentiated cells in the claimed methods. It is only disclosed as one advantageous aspect of being able to use proliferating, non-serum starved cells. Indeed, as discussed beginning at the bottom of page 32 of the specification, only a single mammalian cell is required for the nuclear transfer procedure. Applicants believe that these points are made further clear by the disclosure at page 39, lines 16-19, where it states that "the present invention is *advantageous* in that transgenic procedures can be *simplified* by working with a differentiated cell source that can be clonally propagated."

Thus, it is clear from the disclosure that the cells used as nuclear donors for the claimed methods may be, but not necessarily so, expanded in culture. It is also clear that applicants do not use the term "proliferating" to mean clonally propagated in culture. In fact, even the Examiner acknowledges on page 2 of the Office Action (next to last sentence on the page) that expansion in culture is only "one of many ways" that a cell proliferates, and indeed this is true. Thus, given these observations, it is not clear why the Examiner believes that there is something in the culture expansion procedure that is required for reprogramming of the nucleus after nuclear transfer.

In this regard, it is also not clear to applicants how the fact that the art "as a whole" teaches that some external stimulus is responsible for reprogramming of the nucleus translates into the argument that it must be something in the cell culture procedure solely because this is allegedly the "only discernable treatment" disclosed in the specification. This

is entirely untrue because in fact the "external stimulus" that provides for reprogramming is the oocyte activation procedure following nuclear transfer, not the manner in which the nuclear donor cells are treated prior to nuclear transfer. This is made quite clear in Dr. Robl's declaration filed on January 10, 2000 (see paragraphs 20-22), this is known to those of skill in the art, and moreover this is supported by the observation that Roslin's donor cells, which are serum-starved and therefore certainly not growing in culture, may be used for nuclear transfer.

Thus, it appears that the present rejection stems more from a misunderstanding as to the meaning of the term "proliferating" and its significance to the claimed methods rather than lack of enablement per se. Applicants respectfully submit that the rejection should therefore be withdrawn in view of the above arguments. Applicants would be glad to submit further declaratory evidence confirming that proliferating, non-quiescent cells that are not expanded in culture may be used for nuclear transfer if the Examiner believes such evidence to be necessary. Never-the-less, in order to advance prosecution and ease any concerns the Examiner may have regarding the term "proliferating," the instant claims have been amended to refer to "non-quiescent" cells instead. Reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph is respectfully requested.

Next, claim 109 was rejected as being indefinite under 35 U.S.C. §112, second paragraph because it is a duplicate of claim 108. Applicants respectfully submit that claim 109 was canceled by way of amendment above, and that this rejection has been rendered moot. Withdrawal of the rejection is respectfully requested.

Applicants acknowledge with appreciation the indication that the present claims are free of the prior art. It appears that a Notice of Allowance is next in order. If there are any

further issues to be resolved prior to allowance, the Examiner is respectfully requested to telephone the undersigned so that prosecution may be expedited.

Respectfully submitted,

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proximal biologically active metabolite. Treatment of oocytes with progesterone, the physiological inducer of oocyte maturation, resulted in a time- and concentration-dependent increase in the mass of ceramide and decrease in the mass of sphingomyelin through activation of a Mg(2 +)-dependent neutral sphingomyelinase. These observations suggest that the generation of ceramide from sphingomyelin is part of the signal transduction pathway activated in response to progesterone and that the increase in ceramide is likely to be functionally important in resumption of the meiotic cell cycle.

TITL: Calcium oscillations and protein synthesis inhibition synergistically activate mouse oocytes.

AUTH: Bos-Mikich A; Swann K; Whittingham DG

ORGA: MRC Experimental Embryology and Teratology Unit, St. George's Hospital Medical School, London, UK.

CITE: Mol Reprod Dev 1995 May; 41 (1): 84-90

LANG: ENG; English

ABST: We have examined the ability of the two parthenogenetic agents, strontium (Sr2+) and cycloheximide, to activate mouse oocytes. We demonstrate that Sr2+ and cycloheximide act synergistically to promote parthenogenetic activation up to the pronuclear stage in oocytes collected immediately after ovulation. These two agents appeared to act independently, since incubation in Sr2+ media triggered a series of intracellular Ca2+ rises without affecting protein synthesis and cycloheximide inhibited protein synthesis without causing any intracellular Ca2+ changes. In addition, cycloheximide did not alter the pattern of Ca2+ oscillations induced by Sr2+. In contrast, we show that another commonly used parthenogenetic activation treatment, the Ca2+ ionophore A23187, has dual effects. Exposure of oocytes to the Ca2+ ionophore, A 23187, in Ca(2+)- and Mg(2 +)-free medium leads to the activation of young oocytes. However, as well as generating a Ca2+ increase, the treatment of mouse oocytes with A23187 and Ca(2+)- and Mg(2 +)-free media led to a marked inhibition of protein synthesis. Our data show that parthenogenetic agents may have two important loci for activating mammalian oocytes and that the combined effect on Ca2+ release and protein synthesis is most effective.

TITL: [Oocyte maturation and activation in the common frog and the clawed toad under the action of divalent cations]. Sozrevanie i aktivatsiia ootsitov travianoi i shportsevoi liagushki pod deistviem dvukhvalentnykh ionov.

AUTH: Nikiforova GP; Skoblina MN

CITE: Ontogenez 1992 Nov-Dec; 23 (6): 644-9

LANG: RUS; Russian

ABST: Maturation of Rana temporaria and Xenopus laevis oocytes was induced by solutions containing Mn2+ and Co2+ ions. Completion of oocyte maturation was estimated by the following criteria: (1) appearance of the maturation promoting factor (MPF) in the oocyte cytoplasm and (2) oocyte capacity to activation and formation of male pronuclei from the injected sperm nuclei. X. laevis oocytes matured under the effect of Co2+ ions were shown to contain MPF. Oocytes of both species matured under the effect of either ions could not be activated by pricking with a needle and injected sperm nuclei didn't transform into pronuclei. R. temporaria oocytes matured under the effect of ions in late spring, when natural spawning takes place, showed spontaneous activation.

TITL: Ceramide triggers meiotic cell cycle progression in Xenopus oocytes. A potential mediator of progesterone-induced maturation.

AUTH: Strum JC; Swenson KI; Turner JE; Bell RM

ORGA: Department of Molecular Cancer Biology, Duke University Medical Center, Durham, North Carolina 27710, USA.

CITE: J Biol Chem 1995 Jun 2; 270 (22): 13541-7

LANG: ENG; English

ABST: The role of sphingomyelin-derived second messengers in progesterone-induced reinitiation of the meiotic cell cycle of Xenopus laevis oocytes was investigated. A brief treatment of defolliculated oocytes with sphingomyelinase (Staphylococcus aureus) was sufficient to induce maturation as measured by H1 kinase activity and germinal vesicle breakdown (GVBD). Pretreatment with cycloheximide inhibited sphingomyelinase-induced GVBD demonstrating a requirement for protein synthesis. Microinjection of ceramide or sphingosine, potential products of sphingomyelin hydrolysis, were capable of inducing GVBD in the absence of hormone. Metabolic labeling studies suggested the conversion of sphingosine to ceramide was necessary for sphingosine-induced GVBD. Additionally, fumonisin b1, an inhibitor of sphingosine N-acyltransferase, blocked sphingosine-induced GVBD demonstrating that ceramide is the more